

What is claimed is:

1. A method of treating a mammal that does not have severe combined immune deficiency but is deficient in CD4<sup>+</sup> lymphocytes, the method comprising inoculating the mammal with an attenuated mycobacterium in the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, the mycobacterium comprising two deletions, wherein a virulent mycobacterium in the *M. tuberculosis* complex having either deletion exhibits attenuated virulence.
2. The method of claim 1, wherein the mammal is not deficient in CD8<sup>+</sup> lymphocytes.
3. The method of claim 1, wherein the mammal is deficient in CD8<sup>+</sup> lymphocytes.
4. The method of claim 1, wherein the mammal is at risk for infection by a virulent mycobacterium in the *M. tuberculosis* complex.
5. The method of claim 1, wherein the attenuated mycobacterium is an *M. tuberculosis*.
6. The method of claim 5, wherein the attenuated *M. tuberculosis* is an H37Rv strain.
7. The method of claim 5, wherein the attenuated *M. tuberculosis* is a CDC1551 strain.
8. The method of claim 1, wherein the attenuated mycobacterium is an *M. bovis*.
9. The method of claim 1, wherein the mammal is a cow.
10. The method of claim 1, wherein the mammal is a human.
11. The method of claim 1, wherein the mammal is a human child.
12. The method of claim 1, wherein at least one of the two deletions is a deletion of a region selected from the group consisting of an *RD1* region, a region controlling production of a vitamin, and a region controlling production of an amino acid.
13. The method of claim 12, wherein the deletion is of the *RD1* region.
14. The method of claim 13, wherein the deleted *RD1* region has at least 95% homology to SEQ ID NO:1.
15. The method of claim 13, wherein the deleted *RD1* region comprises SEQ ID NO:1.
16. The method of claim 12, wherein the deletion is of a region controlling production of a vitamin.
17. The method of claim 16, wherein the vitamin is pantothenic acid or nicotinamide adenine dinucleotide (NAD).
18. The method of claim 17, wherein the vitamin is pantothenic acid.

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19. The method of claim 18, wherein the deletion is a  $\Delta panCD$  deletion.
20. The method of claim 19, wherein the  $\Delta panCD$  deletion has at least 95% homology to SEQ ID NO:2.
21. The method of claim 19, wherein the  $\Delta panCD$  deletion comprises SEQ ID NO:2.
- 5 22. The method of claim 12, wherein the deletion is in a region controlling production of an amino acid.
23. The method of claim 22, wherein the amino acid is selected from the group consisting of proline, tryptophan, leucine or lysine.
24. The method of claim 22, wherein the amino acid is lysine.
- 10 25. The method of claim 24, wherein the deletion is a  $\Delta lysA$  deletion.
26. The method of claim 25, wherein the  $\Delta lysA$  deletion has at least 95% homology to SEQ ID NO:4.
27. The method of claim 25, wherein the  $\Delta lysA$  deletion comprises SEQ ID NO:4.
28. The method of claim 12, wherein one deletion is of an *RD1* region and the other
- 15 deletion is of a region that controls production of a vitamin.
29. The method of claim 28, wherein the deletion of the *RD1* region comprises SEQ ID NO:1 and the deletion of a region that controls production of a vitamin comprises SEQ ID NO:2.
30. The method of claim 12, wherein one deletion is of an *RD1* region and the other
- 20 deletion is of a region that controls production of an amino acid.
31. The method of claim 30, wherein the deletion of the *RD1* region comprises SEQ ID NO:1 and the deletion of a region that controls production of an amino acid comprises SEQ ID NO:4.
- 25 32. The method of claim 12, wherein one deletion is of a region that controls production of a vitamin and the other deletion is of a region that controls production of an amino acid.
33. The method of claim 32, wherein the deletion of a region that controls production of a vitamin comprises SEQ ID NO:2 and the deletion of a region that controls production of an amino acid comprises SEQ ID NO:4.
- 30 34. The method of claim 1, wherein at least one of the deletions is made by genetic engineering.
35. The method of claim 1, wherein at least one of the deletions is made by specialized transduction.

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36. The method of claim 1, wherein at least one of the deletions is made by sequential two-step recombination.
37. The method of claim 36, wherein the sequential two-step recombination uses a *sacB* selective marker.
- 5 38. The method of claim 1, wherein the attenuated mycobacterium further comprises a foreign DNA stably integrated into genomic DNA of the mycobacterium.
39. The method of claim 38, wherein the foreign DNA encodes at least one protein or polypeptide selected from the group consisting of an antigen, an enzyme, a lymphokine, an immunopotentiator, and a reporter molecule.
- 10 40. The method of claim 39, wherein the foreign DNA encodes at least one protein antigen selected from the group consisting of antigens from *Mycobacterium leprae*, *Mycobacterium tuberculosis*, malaria sporozoites, malaria merozoites, diphtheria toxoid, tetanus toxoids, *Leishmania* spp., *Salmonella* spp., *Mycobacterium africanum*, *Mycobacterium intracellulare*, *Mycobacterium avium*, *Treponema* spp., Pertussis, Herpes virus, Measles virus, Mumps virus, *Shigella* spp., *Neisseria* spp., *Borrelia* spp., rabies, polio virus, Human immunodeficiency virus, snake venom, insect venom, and *Vibrio cholera*; steroid enzymes; interleukins 1 through 7; tumor necrosis factor  $\alpha$  and  $\beta$ ; interferon  $\alpha$ ,  $\beta$ , and  $\gamma$ ; and reporter molecules luciferase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase and catechol dehydrogenase.
- 15 41. A method of treating a mammal that does not have severe combined immune deficiency but is deficient in CD8 $^{+}$  lymphocytes, the method comprising inoculating the mammal with an attenuated mycobacterium in the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, the mycobacterium comprising two deletions, wherein a virulent mycobacterium in the *M. tuberculosis* complex having either deletion exhibits attenuated virulence.
- 20 42. The method of claim 41, wherein the mammal is not deficient in CD4 $^{+}$  lymphocytes.
43. The method of claim 41, wherein the mammal is deficient in CD4 $^{+}$  lymphocytes.
44. Use of an attenuated mycobacterium in the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex for the manufacture of a medicament for treatment of a mammal that
- 30 45. The use of claim 44, wherein the mammal is not deficient in CD8 $^{+}$  lymphocytes.

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46. The use of claim 44, wherein the mammal is deficient in CD8<sup>+</sup> lymphocytes.
47. The use of claim 44, wherein the mammal is at risk for infection by a virulent mycobacterium in the *M. tuberculosis* complex.
48. The use of claim 44, wherein the attenuated mycobacterium is an *M. tuberculosis*.
- 5 49. The use of claim 48, wherein the attenuated *M. tuberculosis* is an H37Rv strain.
50. The use of claim 48, wherein the attenuated *M. tuberculosis* is a CDC1551 strain.
51. The use of claim 44, wherein the attenuated mycobacterium is an *M. bovis*.
52. The use of claim 44, wherein the mammal is a cow.
53. The use of claim 44, wherein the mammal is a human.
- 10 54. The use of claim 44, wherein the mammal is a human child.
55. The use of claim 44, wherein at least one of the two deletions is a deletion of a region selected from the group consisting of an *RD1* region, a region controlling production of a vitamin, and a region controlling production of an amino acid.
56. The use of claim 55, wherein the deletion is of the *RD1* region.
- 15 57. The use of claim 56, wherein the deleted *RD1* region has at least 95% homology to SEQ ID NO:1.
58. The use of claim 56, wherein the deleted *RD1* region comprises SEQ ID NO:1.
59. The use of claim 55, wherein the deletion is of a region controlling production of a vitamin.
- 20 60. The use of claim 59, wherein the vitamin is pantothenic acid or nicotinamide adenine dinucleotide (NAD).
  61. The use of claim 60, wherein the vitamin is pantothenic acid.
  62. The use of claim 61, wherein the deletion is a *ΔpanCD* deletion.
  63. The use of claim 62, wherein the *ΔpanCD* deletion has at least 95% homology to
- 25 SEQ ID NO:2.
  64. The use of claim 62, wherein the *ΔpanCD* deletion comprises SEQ ID NO:2.
  65. The use of claim 55, wherein the deletion is in a region controlling production of an amino acid.
  66. The use of claim 65, wherein the amino acid is selected from the group consisting
- 30 of proline, tryptophan, leucine or lysine.
  67. The use of claim 65, wherein the amino acid is lysine.
  68. The use of claim 67, wherein the deletion is a *ΔlysA* deletion.

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69. The use of claim 68, wherein the  $\Delta$ lysA deletion has at least 95% homology to SEQ ID NO:4.

70. The use of claim 68, wherein the  $\Delta$ lysA deletion comprises SEQ ID NO:4.

71. The use of claim 55, wherein one deletion is of an RD1 region and the other 5 deletion is of a region that controls production of a vitamin.

72. The use of claim 71, wherein the deletion of the RD1 region comprises SEQ ID NO:1 and the deletion of a region that controls production of a vitamin comprises SEQ ID NO:2.

73. The use of claim 69, wherein one deletion is of an RD1 region and the other 10 deletion is of a region that controls production of an amino acid.

74. The use of claim 73, wherein the deletion of the RD1 region comprises SEQ ID NO:1 and the deletion of a region that controls production of an amino acid comprises SEQ ID NO:4.

75. The use of claim 55, wherein one deletion is of a region that controls production of 15 a vitamin and the other deletion is of a region that controls production of an amino acid.

76. The use of claim 75, wherein the deletion of a region that controls production of a vitamin comprises SEQ ID NO:2 and the deletion of a region that controls production of an amino acid comprises SEQ ID NO:4.

77. The use of claim 44, wherein at least one of the deletions is made by genetic 20 engineering.

78. The use of claim 44, wherein at least one of the deletions is made by specialized transduction.

79. The use of claim 44, wherein at least one of the deletions is made by sequential two-step recombination.

25 80. The use of claim 79, wherein the sequential two-step recombination uses a *sacB* selective marker.

81. The use of claim 44, wherein the attenuated mycobacterium further comprises a foreign DNA stably integrated into genomic DNA of the mycobacterium.

30 82. The use of claim 81, wherein the foreign DNA encodes at least one protein or polypeptide selected from the group consisting of an antigen, an enzyme, a lymphokine, an immunopotentiator, and a reporter molecule.

83. The use of claim 82, wherein the foreign DNA encodes at least one protein antigen selected from the group consisting of antigens from *Mycobacterium leprae*, *Mycobacterium*

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*tuberculosis*, malaria sporozoites, malaria merozoites, diphtheria toxoid, tetanus toxoids, *Leishmania spp.*, *Salmonella spp.*, *Mycobacterium africanum*, *Mycobacterium intracellulare*, *Mycobacterium avium*, *Treponema spp.*, Pertussis, Herpes virus, Measles virus, Mumps virus, *Shigella spp.*, *Neisseria spp.*, *Borrelia spp.*, rabies, polio virus, Human immunodeficiency

5 virus, snake venom, insect venom, and *Vibrio cholera*; steroid enzymes; interleukins 1 through 7; tumor necrosis factor  $\alpha$  and  $\beta$ ; interferon  $\alpha$ ,  $\beta$ , and  $\gamma$ ; and reporter molecules luciferase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase and catechol dehydrogenase.

84. Use of an attenuated mycobacterium in the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex for the manufacture of a medicament for treatment of a mammal that
- 10 does not have severe combined immune deficiency but is deficient in CD8 $^{+}$  lymphocytes, the mycobacterium comprising two deletions, wherein a virulent mycobacterium in the *M. tuberculosis* complex having either deletion exhibits attenuated virulence.
85. The use of claim 84, wherein the mammal is not deficient in CD4 $^{+}$  lymphocytes.
86. The use of claim 84, wherein the mammal is deficient in CD4 $^{+}$  lymphocytes.